Figure 1. Nucleotide and amino acid sequence of 60kCRMP from Chalmydia muridium.

| Met | - | | | Asp | | _ | | | Leu | _ | _ | _ | _ | gtg Val | _ | 48 |
|-----|------------|------|------------|------------|-----|------------|------------|------------|------------|-----|------------|------------|-----|------------|------|-------|
| 1 | | | | 5 | | | | | 10 | | | | | 15 | | |
| | | | | | | | | | | | | | | gtg Val | | 96 |
| | • | | 20 | | | | | 25 | | | | • | .30 | | | |
| | | A | | | Ψ | | | | | | | | | | | |
| | | | | | | | | | | | | | | tta Leu | | 144 |
| | | 35 | | | | | 40 | | | | | 45· | | | | |
| | | | | | | | | | | | | | | aaa | | 192 |
| Asp | Thr 50 | Lys | Ala | Lys | Glu | Thr 55 | Thr | Ser | His | Gln | Lys 60 | Asp | Arg | Lys | Ala | |
| aga | aaa | aat | cat | caa | aat | agg | act | tcc | αta | atc | cat | 222 | σaσ | gtt | act | 240 |
| | | | | | | | | | - | - | _ | | | Val | | |
| gca | att | cat | gat. | act | aaa | act. | gta | gag | cct | aga | caq | gat | tet | tgc | ttt | 288 |
| | | | | Thr | | | | | Pro | | | | | Cys | | 200 |
| | | | | 85 | | | | | 90 | | | | | 95 | | • |
| | | | | | | | | | | | | | | gaa | | . 336 |
| GIY | гÀЗ | Met. | 19r 100 | Inr | vaı | гÀЗ | vaı | Asn 105 | Asp | Asp | Arg | Asn | 110 | Glu | Ile | |
| | | | | | | | | | | | | | | cct | | 384 |
| Vạl | Gln | Ser | ۷al | Pro | Glu | Tyr | Ala 120 | Thr | Val | Gly | Ser | Pro 125 | Tyr | Pro | Ile | |
| | | | | | | | | | | | • | | | | | |
| _ | | | | | | | _ | - | _ | _ | _ | - | | att Ile | | 432 |
| | 130 | | ., | | 1 | 135 | 9 | | 0,70 | | 140 | ,,,, | | | | |
| caq | caa | tta | cca | tac | gaa | aca | gag | ttt | att | agc | agt. | σat | cca | gct | act | 480 |
| | _ | | | _ | - | - | | | _ | _ | _ | _ | | Ala | | |
| 145 | | | | | 150 | ٠ | | | | 155 | | | | | 160 | |
| | | | | | | | | | | | | | | tta | | 528 |
| Thr | Pro | Thr | Ala | Asp 165 | Gly | Lys | Leu | Val | Trp 170 | Lys | Ile | Asp | Arg | Leu 175 | Gly | |
| | | | | 105 | | | | | | | | | | | | |
| | | _ | _ | - | | | | _ | | - | | | | aaa Lys | _ | 576 |
| Gin | GLY | GIU | 180 | per | ъўs | 116 | 1111 | 185 | пр | vaı | пуз | PIO | 190 | пуь | GIU. | |
| qqt | tgc | tac | ttt | aca | act | qca | acq | att | tat | act | tat | cca | gag | atc | cat | 624 |
| | | Cys | | | | | Thr | | | | | Pro | | Ile | | |
| | | 195 | | | | | 200 | | | | | 205 | | | | |
| | | | | | | | | | | | | | | gaa | | 672 |
| Ser | Val 210 | Thr | гла | Сув | Gly | Gln 215 | Pro | Ala | Ile | Сув | Val 220 | Lys | Gln | Glu | Gly | |
| | | | | | | | | | | | | | | | | |
| | _ | _ | _ | - | - | - | _ | | _ | | | _ | | aat Asn | _ | 720 |
| 225 | JAU | | | Cys | 230 | ~-3 | -yo | | *41 | 235 | -1- | ~~ A | *** | 1.011 | 240 | |

| | | | | • | | | | | | | | | | | | |
|---|---|---|---|---|-------------------|---|-----|---|---|---|---|---|---|---|------------|------------------|
| _ | | | | | gca Ala | | _ | _ | | _ | - | | _ | | | 768 |
| | | | | | gct Ala | | | | | | | | | | | 816 |
| | | | _ | _ | caa Gln | | | _ | - | - | | | | | - | 864 |
| | _ | - | | | cgt Arg | | Arg | | | | | _ | | _ | | 912 |
| | | | | | aaa Lys 310 | | | | | | | | | | | 960 _. |
| | | | | | gtt Val | | | | | | | | | | gtt Val | 1008 |
| | | | | - | tat Tyr | | | | _ | | | | | - | | 1056 |
| - | | | | _ | gta Val | | - | - | | | | | | | | 1104 |
| _ | | | | | gga Gly | _ | - | | | _ | | | _ | | | 1152 |
| | | | | | aat Asn 390 | | | | | | | | _ | _ | | 1200 |
| | | | | | cca Pro | | | | | | | | | | | 1248 |
| _ | _ | | - | _ | ggt Gly | | _ | | | _ | _ | - | _ | | | 1296 |
| | | | | | gct Ala | | | | | | | | | | | 1344 |
| | | | | | gga Gly | | | | | | | | | | | 1392 |
| | | | | | gaa Glu 470 | | | | | | | | | | | 1440 |
| | | | | | cct Pro | | | | | | | | | | | 1488 |

| | | | | 485 | | | | | 490 | | | | | 495 | | |
|-------------------|-------------------|-------------------|--------------------|------------|-------------------|-------------------|-------------------|-------------------|------------|------------|-------------------|-------------------|-------------------|------------|------------|------|
| att Ile | aca Thr | gga Gly | aac Asn 500. | Thr | gta Val | gtg Val | ttt Phe | gat Asp 505 | tcg Ser | tta Leu | cct Pro | aga Arg | tta Leu 510 | ggt Gly | tct Ser | 1536 |
| aaa Lys | gaa Glu | act Thr 515 | gta Val | gag Glu | ttt Phe | tct Ser | gta Val 520 | acg Thr | ttg Leu | aaa Lys | gca Ala | gta Val 525 | tcc Ser | gct Ala | gga Gly | 1584 |
| gat Asp | gct Ala 530 | cgt Arg | 999 999 | gaa Glu | gct Ala | att Ile 535 | ctt Leu | tct Ser | tcc Ser | gat Asp | aca Thr 540 | ttg Leu | aca Thr | gtt Val | cct Pro | 1632 |
| gta Val 545 | tct Ser | gat Asp | acg Thr | gag Glu | aat Asn 550 | aca Thr | cat His | atc Ile | tat Tyr | | | | | | | 1662 |

Figure 2 C. trachomatis equivalent 60kCRMP nucleic acid and amino acid sequence.

| ato Met | g cga : Arc | ata Ile | gga Gly | gat Asp | cct Pro | atg Met | aac Asn | aaa Lys | cto Leu | ato | aga Arg | ı cga | gca | Val | acg Thr | | 48 |
|-------------------|-------------------|-------------------|-------------------|------------------|------------------------|-------------------|-------------------|-------------------|------------------|-------------------|-------------------|-------------------|-------------------|------------------|-------------------|---|-----|
| ato Ile | ttc Phe | gcg Ala | gtg Val | act Thr | agt Ser | gtg Val | gcg Ala | agt Ser 25 | : tta | ttt Phe | gct Ala | ago Ser | 999 30 | 15 gtc Val | tta Leu | | 96 |
| gag Glu | acc Thr | tct Ser 35 | atg Met | gca Ala | ↓ gag Glu | tct Ser | ctc Leu 40 | tct Ser | aca Thr | aac Asn | gtt Val | att Ile 45 | ·ago | tta Leu | gct Ala | | 144 |
| gac | acc Thr | aaa Lys | gcg Ala | aaa Lys | gac Asp | aac Asn 55 | act Thr | tct Ser | cat His | aaa Lys | ago Ser 60 | aaa Lys | aaa Lys | gca Ala | aga Arg | | 192 |
| aaa Lys 65 | aac | cac His | agc Ser | aaa Lys | gag Glu 70 | act Thr | ccc Pro | gta Val | gac Asp | cgt Arg 75 | aaa Lys | gag Glu | gtt Val | gct Ala | ccg Pro 80 | | 240 |
| gtt Val | cat His | gag Glu | tct Ser | aaa Lys 85 | gct Ala | aca Thr | gga Gly | cct Pro | aaa Lys 90 | cag Gln | gat Asp | tct Ser | tgc Cys | ttt Phe 95 | ggc | | 288 |
| aga Arg | atg Met | tat Tyr | aca Thr 100 | gtc Val | aaa Lys | gtt Val | aat Asn | gat Asp 105 | gat Asp | cgc Arg | aat Asn | Val | gaa Glu 110 | atc Ile | aca Thr | • | 336 |
| caa Gln | gct Ala | gtt Val 115 | cct Pro | gaa Glu | tat Tyr | gct Ala | acg Thr 120 | gta Val | gga Gly | tct Ser | ccc Pro | tat Tyr 125 | cct Pro | att Ile | gaa Glu | | 384 |
| att Ile | act Thr 130 | gct Ala | aca Thr | ggt Gly | aaa Lys | agg Arg 135 | gat Asp | tgt Cys | gtt Val | gat Asp | gtt Val 140 | atc Ile | att Ile | act Thr | cag. Gln | | 432 |
| 145 | ьeu | Pro | Cys | Glu | gca Ala 150 | Glu | Phe | Val | Arg | Ser 155 | Asp | Pro | Ala | Thr | Thr 160 | | 480 |
| Pro | rnr | Ala | Asp | Gly 165 | aag Lys | Leu | Val | Trp | Lys 170 | Ile | Asp | Arg | Leu | Gly 175 | Gln | | 528 |
| GIĀ | GIU | гÀв | 180 | Lys | att Ile | Thr | Val | Trp 185 | Val | ГУS | Pro | Leu | Lys 190 | Glu | Gly | | 576 |
| Cys | Cys | 195 | Tnr | | gca Ala | Thr | Val 200 | Сув | Ala | Суз | Pro | Glu 205 | Ile | Arg | Ser | | 624 |
| vaı | 210 | rys | Сув | GТÀ | | Pro 215 | Ala | Ile | Сув | Val | Lys 220 | Gln | Glu | Gly | Pro | | 672 |
| gag Glu 225 | aat Asn | gct Ala | tgt Cys | ttg Leu | cgt Arg 230 | tgc Cys | cca Pro | gta Val | gtt Val | tac Tyr 235 | aaa Lys | att Ile | aat Asn | ata Ile | gtg Val 240 | | 720 |

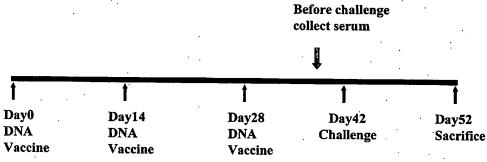
| | | gga Gly | | | | | | | | | | | | | | 768 |
|-------------------|-------------------|-------------------|-------------------|------------|-------------------|-------------------|------------|-------------------|------------|-------------------|-------------------|------------|-------------------|------------|-------------------|------|
| | | ggt Gly | | | | | | | | | | | | | | 816 |
| | | gat Asp 275 | | | | | | | | | | | | | | 864 |
| | | ctt Leu | | | | | | | | | | Thr | | | | 912 |
| , | _ | gga Gly | | | | | _ | | _ | | | | | | | 960 |
| | | gta Val | | | | | | | | | | | | | | 1008 |
| aag Lys | cct Pro | gta Val | gaa Glu 340 | tat Tyr | gtg Val | atc Ile | tcc Ser | gtt Val 345 | tcc Ser | aat Asn | cct Pro | gga Gly | gat Asp 350 | ctt Leu | gtg Val | 1056 |
| | | gat Asp 355 | | | | | | | | | | | | | | 1104 |
| | | gct Ala | | | | | | | | | | | | | | 1152 |
| gtg Val 385 | aaa Lys | gaa Glu | ctg Leu | aat Asn | cct Pro 390 | gga Gly | gag Glu | tct Ser | cta Leu | cag Gln 395 | tat Tyr | aaa Lys | gtt Val | cta Leu | gta Val 400 | 1200 |
| | | caa Gln | | | | | | | | | | | | | | 1248 |
| | | gac Asp | | | | | | | | | | | | | | 1296 |
| | | gga Gly 435 | | | | | | | | | | | | | | 1344 |
| cct Pro | gtt Val 450 | tgt Cys | gta Val | gga Gly | gaa Glu | aat Asn 455 | act Thr | gtt Val | tac Tyr | cgt Arg | att Ile 460 | tgt Cys | gtc Val | acc Thr | aac Asn | 1392 |
| aga Arg 465 | ggt Gly | tct Ser | gca Ala | gaa Glu | gat Asp 470 | aca Thr | aat Asn | gtt Val | tct Ser | tta Leu 475 | atg Met | ctt Leu | aaa Lys | ttc Phe | tct Ser 480 | 1440 |
| | | ctg Leu | | | | | | | | | | | | | | 1488 |

| | | | | 485 | | | 490 | | | | 495 | | |
|---|-----|---|-------------------|-----|---|---|-----|---|---|---|-----|---|------|
| | | | aca Thr 500 | | | | | | | | | | 1536 |
| _ | | _ | gag Glu | | _ | _ | | _ | _ | _ | | _ | 1584 |
| | | | gaa Glu | | | | | | | | | | 1632 |
| | Asp | | gag Glu | | | | | | | | • | | 1659 |

Figure 3 Immunization protocol.

Protocol

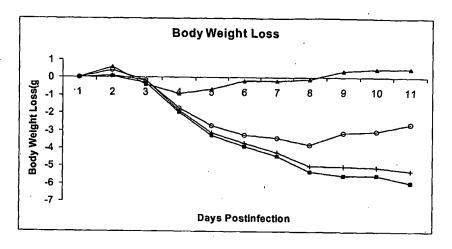
Animal: Female BALB/c mice(4-5weeks old)
: Four to 8 mice per group



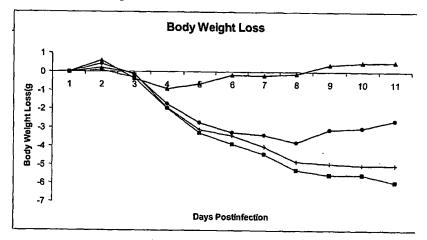
DNA Vaccine: Intranasal 100ug&intramuscular 200ug of plasmid DNA(2ug/ul)

Figure 4 Body Weight loss after immunization.

Panel A 60kCRMP full-length



Panel B 60kCRMP signal sequence deleted

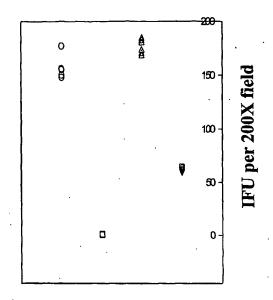


▲ - EB

- o-pCACT CRMP60k
- - pCACT CRMPdelta
- +-pCAMycHis
- Naive

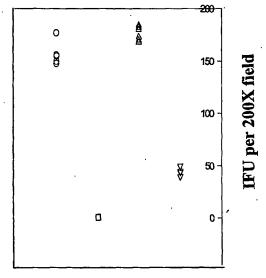
Figure 5 Clearance of Chlamydia from the Lungs of Immunized Mice.

Panel A 60kCRMP full-length



(p<0.001)

Panel B 60kCRMP signal sequence deleted



(p<0.001)

o- Naïve,

 \Box – EB,

 Δ - pCAMycHis,

∇ - pCACT CRMP60k (Panel A)

- pCACT CRMPdelta

Figure 6. Plasmid pET30b(+)60kCRMP+SP cloning Procedure.

